学校编码：10384　　分类号＿密级
学号：31420081150630　　UDC＿＿

厦门大学

硕 士 学 位 论 文

甲亢和 Wilson 病的核磁共振代谢组学方法研究

Studies on Hyperthyroidism and Wilson's Disease based on NMR Metabolomics

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专 业 名 称：生物医学工程
论文提交日期：2011 年 05 月
论文答辩时间：2011 年　　月
学位授予日期：2011 年　　月

答辩委员会主席：＿
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2011 年　5 月

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中 文 摘 要

代谢组学方法是上世纪 90 年代中期发展起来的一门新学科，它借助高通量、高灵敏度与高精确度的现代分析技术，分析细胞、组织和生物体液中内源性代谢物的整体组成，并通过代谢物复杂的、动态的变化，辨识和解析被研究对象的生理病理状态。本文将核磁共振代谢组学方法应用于两种代谢性疾病的研究中，甲状腺功能亢进是一种代谢性增强的疾病，Wilson 病(Wilson's disease, WD)是机体内对铜代谢不足导致铜在体内大量积累的疾病。运用核磁共振代谢组学研究方法，分析两种代谢性疾病所导致的代谢改变，寻找了甲亢和 Wilson 病的特征代谢物。对甲亢疾病的研究中，进一步分析了家族性甲亢的代谢差异。对 Wilson 病的研究中，综合探讨了 Wilson 病早期、模型期以及青霉胺药物干预后的代谢差异，为 Wilson 病的早期诊断和治疗以及药物疗效的评价提供科学依据。本论文的主要内容归纳如下：

一、简要介绍了基于 NMR 的代谢组学方法及其应用。概述了 NMR 代谢组学方法在疾病研究方面的进展。

二、总结并分析 NMR 代谢组学中常用的生物样品的处理方法，主要包括血液、尿液和生物组织样品。讨论这些生物样品的理化性质、内源性代谢物、样品的采集、保存以及实验样品的配制等。

三、应用高分辨液体 ^{1}H NMR 谱学结合模式识别方法，研究了甲亢患者的血清和尿液。结果表明，与正常人相比，甲亢患者血清中，胆碱、葡萄糖、三甲胺等物质的含量升高，而 VLDL、LDL、胆固醇等脂质，以及乳酸、糖蛋白、丙氨酸等代谢物的含量下降；甲亢患者尿液中的葡萄糖、柠檬酸、牛磺酸以及肌氨酸等代谢物的含量升高，而马尿酸、TMAO、甲酸、琥珀酸等代谢物的含量下降。由于甲亢疾病有一定的家族遗传性，进一步研究了家族遗传性甲亢代谢差异，寻找家族遗传性甲亢的代谢改变。

四、应用高分辨液体 ^{1}H NMR 谱学结合多元数据分析方法，研究了 Wilson 病模型大鼠早期、模型期以及青霉胺药物干预后代谢变化。WD 模型组中，血清中的乳酸、肌酸、肌酐、亮氨酸、异亮氨酸含量显著升高，葡萄糖、TMAO、肌醇和甘氨酸等物质的含量下降；WD 组尿液中，丙酮、肌酸、肌酐以及三羧酸循

环中间产物以及酮体物质的含量升高，葡萄糖、甘氨酸的含量下降。血清中显著升高的乳酸和尿液中的大量增加的乙酸表明 Wilson 病导致糖代谢和能量代谢的紊乱。尿液中增加的酮体物质，改变的丙氨酸和葡萄糖表明 WD 大鼠中肝脏受到了损伤。血清和尿液中显著升高的肌酸和肌酐是肾脏受损的标志。血清和尿液中胆碱及其代谢产物甜菜碱含量的减少表明胆碱代谢发生了紊乱。与 WD 模型组相比，青霉胺干预后大鼠的代谢发生了显著的变化。这些改变表明药物干预后 WD 大鼠的受损各项功能有一定程度的恢复。此外，我们还研究了 WD 病早期代谢显型，发现了 WD 早期的特征代谢物。这些研究结果表明，基于 NMR 代谢组学分析方法在疾病的研究中能够提供更加深入的信息，有广泛的应用前景。

关键词：核磁共振的代谢组学；甲状腺功能亢进；Wilson病

ABSTRACT

The quantitative measurement of the dynamic multi-parametric metabolic response of a living system to pathophysiological stimuli or genetic modification is termed “metabolomics”. The ^{1}H NMR spectroscopy has been shown to be one of the most important analytical techniques used in metabolomics, as it can detect many endogenous metabolites rapidly and reproducibly without tendentiousness or separation. Nuclear magnetic resonance (NMR)-based metabonomics was applied to study the hyperthyroidism and Wilson’s disease by analyzing metabolic profiling of serum and urine. The purpose of this study is to determine an array of characteristic metabolites in serum and urine samples from hyperthyroidism and Wilson’s disease based on NMR spectroscopy. The metabolic changes of familial hyperthyroidism patients were investigated. Besides, we have used the copper-overloaded rats model for Wilson’s disease (WD) combining with the ^{1}H NMR spectroscopy to characterize the alteration of metabolic profiles for the early stage, the later stage of disease and the penicillamine treated group. The main works are summarized as follows:

1. The concepts of NMR-based metabolomics were introduced briefly. An evaluation of progress to date in application of NMR-based metabolomics for the understanding of pathogenic mechanism was reviewed.

2. The collection, preparation and analysis by NMR spectroscopy of serum, urine and tissue samples were summarized. The physical and chemical properties, endogenous metabolites, sample collection, preservation, and NMR experimental preparation of these biological samples were also discussed.

3. High resolution NMR spectroscopy combined with multivariate statistical analysis was applied to investigate the urine and serum of hyperthyroidism patients. Results showed that the elevated levels of choline, glucose and declined levels of VLDL, LDL, cholesterol, lactate, glycoprotein and alanine were detected in serum samples of hyperthyroidism group. In urine samples, the hyperthyroidism group

showed increased levels of glucose, citrate, taurine and creatinine as well as decreased levels of hippurate, trimethylamine-N-oxide, formate and succinate. Furthermore, the metabolic profiling of familial hyperthyroidism patients was also investigated.

4. ^{1}H NMR spectroscopy in combination with multivariate statistical analysis was applied to explore the metabolic profiles from WD model , the early stage of WD model and penicillamine -treated rats using biofluids. WD rats showed increased levels of lactate, creatine, creatinine, leucine, isoleucine and decreased levels of glucose, TMAO, *myo*-inositol and glycine in serum, as well as increased excretion of urinary acetone, creatine, creatinine and Kreb's cycle intermediates, ketone bodies, and decreased levels of urinary glucose, glycine. Significantly elevated levels of lactate in serum and urinary acetate indicated carbohydrate metabolism and energy metabolism disturbance in Wilson's disease. Increased excretion of ketone bodies, altered alanine and glucose metabolites suggested that the liver was possibly impaired in WD rats. Elevated level of creatine and creatinine in serum and urine were observed in WD rats, which could be biomarkers for kidney injury. The decrease of choline and its metabolite betaine in serum and urine indicate a disturbance in choline metabolism. In contrast to the WD group, the metabolite profile of the penicillamine (PA) treated group were affected obviously. These perturbations indicate that the PA-treated group was recovery from WD group in a certain extent. In addition, the metabolic phenotypes of early stage of Wilson's disease rats model were investigated using ^{1}H NMR spectroscopy together with pattern recognition to determine its characteristic metabolites. The study shows the potential application of NMR-based metabonomic analysis in biochemical profile and providing further insight into the molecular mechanism underlying the disorder.

Keywords: NMR-based metabolomics, Hyperthyroidism, Wilson's disease

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